Identification of Genetic Factors Contributing to Heterosis in a Hybrid From Two Elite Maize Inbred Lines Using Molecular Markers

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ABSTRACT

The use of molecular markers to identify quantitative trait loci (QTLs) affecting agriculturally important traits has become a key approach in plant genetics–both for understanding the genetic basis of these traits and to help design novel plant improvement programs. In the study reported here, we mapped QTLs (and evaluated their phenotypic effects) associated with seven major traits (including grain yield) in a cross between two widely used elite maize inbred lines, B73 and Mo17, in order to explore two important phenomena in maize genetics–heterosis (hybrid vigor) and genotype-by-environment (G × E) interaction. We also compared two analytical approaches for identifying QTLs, the traditional single-marker method and the more recently described interval-mapping method. Phenotypic evaluations were made on 3168 plots (nearly 100,000 plants) grown in three states. Using 76 markers that represented 90–95% of the maize genome, both analytical methods showed virtually the same results in detecting QTLs affecting grain yield throughout the genome, except on chromosome *6*. Fewer QTLs were detected for other quantitative traits measured. Whenever a QTL for grain yield was detected, the heterozygote had a higher phenotype than the respective homozygote (with only one exception) suggesting not only overdominance (or pseudo-overdominance) but also that these detected QTLs play a significant role in heterosis. This conclusion was reinforced by a high correlation between grain yield and proportion of heterozygous markers. Although plant materials were grown and measured in six diverse environments (North Carolina, Iowa and Illinois) there was little evidence for G × E interaction for most QTLs.

GENETIC markers have been used to study quantitatively inherited traits for nearly 70 years. SAX (1923) reported the association of seed coat pattern and pigmentation with seed size differences in *Phaseolus vulgaris*. RASMUSSON (1933) and EVERSON and SCHALLER (1955) subsequently reported linkages between single genetic markers and *q*uantitative *t*rait *l*oci (QTLs), and THODAY (1961) greatly elaborated upon the subject. These classical studies employed morphological mutations as genetic markers–which posed major limitations on such research because only a few such markers could be followed in any given cross and because the markers themselves often produced confounding phenotypic effects (TANKSLEY *et al.* 1989; STUBER 1989, 1992). Recently, molecular markers–particularly DNA polymorphisms–have provided geneticists with an essentially unlimited supply of phenotypically neutral markers with which to study the inheritance of quantitative traits and to manipulate these traits for plant and animal improvement. Contributions to the concepts and theory of using mapped genetic markers for identifying, locating and manipulating QTLs have been reviewed in a recent paper on biochemical and molecular markers in plant breeding (STUBER 1992). In principle, the genetic analysis of QTLs should provide both a molecular and a practical understanding of key phenomena in plant improvement.

In this paper, we describe a study using mapped genetic markers to explore two important issues in maize genetics, heterosis and genotype-by-environment (G × E) interaction. Heterosis (or hybrid vigor) is the principal reason for the success of the commercial maize industry. The term, heterosis, was coined by G. H. SCHULL and first proposed in 1914 (see HAYES 1952) and is usually described in terms of the superiority of F_1 hybrid performance over some measure of parental performance. However, the underlying genetic basis for the phenomenon has not been satisfactorily explained. Possible explanations include true overdominance (*i.e.*, single loci at which two alleles have the property that the heterozygote is truly superior to either homozygote), pseudo-overdominance (*i.e.*, nearby loci at which alleles having domi-

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nant or partially dominant advantageous effects are in repulsion linkage phase), or even certain types of epistasis. By mapping QTLs contributing to heterosis in a cross between two inbred maize lines, B73 and Mo17, and evaluating the phenotypic effects associated with these QTLs, our goal was to lay a foundation for understanding the basis for this important phenomenon.

G × E interaction is an essential issue in the assessment of mechanisms of inheritance as well as the prediction of performance in breeding programs because genotypic values must be inferred from phenotypic responses. Clearly, phenotypic performance depends on both genetic and nongenetic influences on plant development. The relative rankings of genotypes may well differ in different environments and the relationship may be quite complex (ALLARD and BRADSHAW 1964). Many quantitative traits in maize, including grain yield, show significant variation attributable to genotype-environmental interactions (MOLL *et al.* 1978). Classical studies on quantitative traits have measured G × E interaction averaged across the entire genome rather than for individual QTLs, while recent studies of QTLs (*e.g.*, EDWARDS, STUBER and WENDEL 1987; STUBER, EDWARDS and WENDEL 1987; ABLER, EDWARDS and STUBER 1991; EDWARDS *et al.* 1992) have focused on mapping QTLs in a fixed environment (but see PATERSON *et al.* 1991). Here, we have attempted to discern the degree of G × E interaction at individual QTLs by first comparing QTL maps generated in six diverse environments. We then contrasted these results with location (environment) by marker interaction variances obtained from traditional analyses of variance.

In addition to these scientific objectives, the current work also had the methodological objective of comparing two different analytical methods for identifying QTLs: the traditional single-marker approach (in which the chromosomal position of the QTL is assumed to lie exactly at the marker locus) and the more recently described interval mapping (LANDER and BOTSTEIN 1989) in which the QTL is taken to lie at its most likely position between two markers flanking an interval.

MATERIALS AND METHODS

Experimental procedures: The experimental materials were developed by first intercrossing two elite maize inbred lines, B73 and Mo17. This cross produces superior hybrid performance and the parental lines, or lines derived from them, have been and are still widely used in commercial hybrids. From this cross, 264 F_3 lines were developed through two selfing generations with each F_3 line tracing to a different F_2 plant. A single plant from each F_3 line was: (1) selfed to produce F_4 progeny, of which approximately 10 were bulked and used to infer the genotype of the F_3 parent, and (2) backcrossed to each of the two parental lines

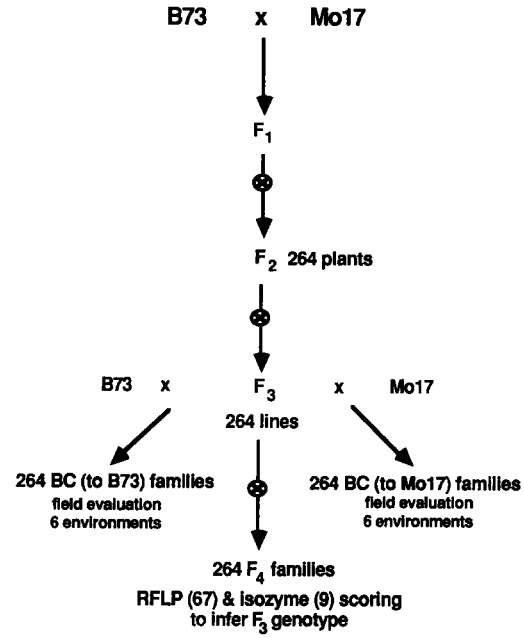

FIGURE 1.—Diagram of procedures for developing, genotyping and phenotyping experimental materials.

to produce progeny which were phenotyped in field evaluations. Figure 1 outlines these procedures.

Genotypes were determined for 9 isozyme loci in the maize isozyme laboratory at Raleigh, North Carolina, using techniques reported by STUBER *et al.* (1988), and for 67 restriction fragment length polymorphism (RFLP) loci at Native Plants, Inc., at Salt Lake City, Utah, using methods reported by HELENTJARIS *et al.* (1985). Identification and distribution of the 76 molecular markers in the maize genome is shown in Figure 2.

Phenotypes were evaluated for each of the 528 (= 264 lines × 2 parents) backcross families by growing 24–35 plants in each of six diverse environments (locations) in 1987–four in North Carolina, one in Iowa, and one in Illinois. Therefore, 3168 plots (nearly 100,000 plants) were evaluated and growing conditions were good at all locations. Field plots were two rows; row length varied from 3.66 to 4.57 m and planting density varied from 36,000 to more than 50,000 plants per hectare (14,600 to more than 20,000 plants per acre). For the field design, the 528 backcross families were subdivided into 22 sets of 24 families each, with each set containing the crosses of 12 randomly chosen F_3 lines to each of the two parental lines, B73 and Mo17. Each family was replicated six times, once in each of the six locations. The quantitative traits measured are listed in Table 1.

Linkage analysis of genetic markers: Although isozyme and RFLP markers used in this study had been mapped previously (STUBER *et al.* 1988; HELENTJARIS, WEBER and WRIGHT 1988), these earlier studies involved different, and usually smaller, populations. Accordingly, genetic maps were calculated from the genotypic data *de novo* and checked for consistency with previously reported maps.

Pairwise and multipoint linkage analyses were performed using a modified version of the MAPMAKER program (LANDER *et al.* 1987) on a Digital DECStation 5000. MAPMAKER's linkage analysis algorithms were modified to allow for correct multipoint maximum likelihood calculation of recombination rates in an F_3 population, *i.e.*, for each locus, probability distributions were calculated over all possible combinations of F_2 and F_3 phase-known genotypes (16

TABLE 1

Means of quantitative traits measured at each location (environment) where data were recorded

Location[a]	Trait[b]						
	Grain yield (bu./acre)	Ear height (m)	Plant height (m)	Ear leaf area (cm^2)	Days to tassel (no.)	Grain moisture (%)	Ears/plant (no.)
1	77.46	0.87	2.59	593.8	65.7	17.6	1.02
3	88.20	1.07	2.74	619.4	64.4	13.0	0.99
4	77.67	0.87	2.39	557.1	66.6	13.7	0.97
5	62.97	0.86	2.36	559.8	68.8	—	0.92
6	109.17	1.07	2.65	—	71.6	21.6	0.85
7	115.54	1.24	2.83	—	—	17.7	—
Mean (over locations)	88.50	1.00	2.59	582.6	67.4	16.7	0.95

[a] 1 = Clayton, North Carolina; 3 = Lewiston, North Carolina; 4 and 5 = Plymouth, North Carolina; 6 = Bloomington, Illinois; 7 = Johnston, Iowa.
[b] "—" indicates that no data were recorded.

per locus). The hidden Markov chain approach was adapted for the expectation step of the *E-M* algorithm (LANDER and GREEN 1987).

Linkage groups were determined using pairwise analyses with a LOD threshold of 4.0. From each linkage group, a subset of well spaced and highly informative markers was chosen, and multipoint analyses comparing thousands of candidate orders were used to determine the most likely framework map (with genetic orders accepted when they had a 1000-fold higher likelihood than alternative orders). Consistency of the data for the framework markers was checked by successively removing markers one at a time from the linkage group and reanalyzing the group's data *de novo*. Significant changes in the map distances or orders were used to detect potential scoring errors. In addition, when final map orders were determined, isolated obligate double crossover events were used to detect further potential data errors. The remaining markers from each linkage group were placed into intervals in the framework using three point analysis and relative orders were determined and confirmed using extensive multipoint analysis involving permutations of loci (at 100:1 likelihood ratio).

QTL mapping using traditional (one-marker-at-a-time) approach: The traditional approach for detecting a QTL near a marker (SOLLER and BRODY 1976; TANKSLEY, MEDINA-FILHO and RICK 1982; EDWARDS, STUBER and WENDEL 1987) involves comparisons among the phenotypic means of appropriate marker classes of progeny. In this study, progeny were generated from backcrossing F_3 lines to the parents, B73 and Mo17. If B_i designates the allele at the ith locus originating from B73 and M_i designates the allele at the ith locus originating from Mo17, then the expected genotypic ratio of the F_3 lines would be 3/8 B_iB_i: 1/4 B_iM_i: 3/8 M_iM_i for the ith locus. To analyze the effect of the ith locus in the backcross to B73 (for example), we compared the phenotypic means of the backcross progeny from F_3 parents having genotype B_iB_i (whose backcross progeny had genotype B_iB_i) to the phenotypic mean of the backcross progeny from F_3 parents having genotype M_iM_i (whose backcross progeny had genotype B_iM_i). Because those F_3 parents with the B_iM_i genotype would produce backcross progeny segregating at this locus, they would not correspond to either cell in a traditional single-marker analysis for the ith locus and so were omitted. [The omission of these progeny represents a limitation of the traditional single-marker approach for the type of experimental materials used in this study, which becomes serious if one wishes to construct multilocus regression models. With a four locus model, one would expect to retain only about 32% (= 0.75^4) of the data. For other types of experiments, such as those using F_2 individuals or recombinant inbred lines, this limitation would not exist for the single-marker analysis.] For this study, about 600 observations were available for computing each of the backcross progeny means used to estimate the effects associated with each marker locus. Therefore, the means were estimated with a high level of precision and the omission of progeny originating from F_3 parents with the B_iM_i genotype was not considered to be a serious limitation for the single marker locus comparisons.

Again, using the backcross to B73 as an example, the difference between the two marker class means provided an estimate of the phenotypic effect of substituting an M allele for a B allele at the QTL associated with the marker. To test the significance of the difference, we performed two different analyses of variance and evaluated the results with an appropriate F-test. First, we performed a simple one-factor ANOVA ignoring location and set effects: each F_3 parent was assigned the mean phenotypic value of its backcross progeny averaged over sets and locations (environments). (It should be noted that F- tests for the one-factor ANOVA were conducted both with and without the B_iM_i genotypic class. Significance levels tended to be lower with the inclusion of the B_iM_i class, however, overall interpretations of the results did not differ.) This analysis was appropriate for comparison with the LOD score analysis (discussed in the next section), inasmuch as the latter analysis also did not account for set and location effects. Second, we performed a three-factor ANOVA that accounted for set and location effects. Because the location (environment) by marker ($L \times M$) and the location by set by marker ($L \times S \times M$) components of variance usually were not significant, the mean square associated with the set by marker ($S \times M$) source of variation was used as the error variance for this F-test (see Table 2a). This is expected to provide a conservative test because both sampling variation (only 12 lines were included in each set) and environmental variation would contribute to the size of this error term.

For each marker, we calculated the following two quantities according to the ANOVA in Table 2:

$$_sR^2 = SS[\text{Markers}]/SS[\text{Families}],$$

and

$$_sR^{*2} = SS[\text{Markers in Sets}]/\{SS[\text{Markers in Sets}] + SS[\text{Families in Markers in Sets}]\}.$$

TABLE 2

ANOVA calculations

Source[a]	d.f.
a) Form of analysis of variance for partitioning variance to test significance of phenotypic effects associated with markers[b]	
Locations (L)	5
Sets (S)	21
Markers (M)	1
$L \times S$	105
$L \times M$	5
$S \times M$	21
$L \times S \times M$	105
Residual	
b) Form of analysis for partitioning variance due to families for computing ${}_sR^2$ (see text)	
Locations	5
Families	263
Locations × families	1315
c) Form of analysis of variance for assessing proportion of genetic variance ${}_sR^{*2}$ ascribed to QTLs associated with specific markers	
Locations (L)	5
Sets (S)	21
Markers (M) in S	22
Families (F) in M in S	Varies
Residual	

[a] Locations, sets and lines were assumed to be random variables; markers were assumed to be fixed.

[b] Significance test for markers: $F_M = M/(S \times M)$. Significance test for locations × markers: $F_{L \times M} = (L \times M)/(L \times S \times M)$.

The quantity ${}_sR^2$ is the proportion of the total phenotypic variance among families (without controlling for effects of locations and sets) that could be ascribed to QTLs associated with an individual marker, while the quantity ${}_sR^{*2}$ reflects the proportion of the variance explained when controlling for the effects of both locations and sets, which more nearly reflects the proportion of genetic variance explained.

QTL analysis using maximum likelihood methods based on interval mapping: Maximum likelihood methods have recently been described (LANDER and BOTSTEIN 1989) that generalize the traditional single-marker analysis to the situation in which the QTL does not lie exactly at the marker locus but rather between two flanking markers and in which some data may be missing. The strength of evidence for linkage is reflected in a LOD score, or logarithm to the base 10 of the likelihood ratio. In this work, interval mapping of QTLs was performed using the basic approach described previously for backcrosses (PATERSON *et al.*, 1988), but using the version of the MAPMAKER-QTL modified for F_3 progeny (with changes as described above). For all traits, analyses were first performed on the untransformed phenotypic data. For those traits that were not approximately normally distributed, the data were also transformed to more closely fit a normal distribution and the analyses were repeated. Such analyses did not substantially alter any of the conclusions and so are not reported.

For the main analysis, each F_3 parent was assigned the mean phenotypic value of its progeny averaged over environments–with set and location effects being ignored (inasmuch as MAPMAKER-QTL does not include an option to account for such effects). Because each F_3 individual's phenotype was defined as the average phenotype of its backcross progeny, the measured QTL effects must necessarily be additive: F_3 individuals heterozygous at any particular QTL necessarily produce backcross families which resemble, on average, a 50–50 mixture of backcrossed families produced by the two types of homozygotes for the QTL. Accordingly, the analysis has 1 d.f. Also, a LOD score threshold of 2.6 corresponds to an approximate nominal significance level of $P = 0.001$ per test, or $P = 0.05$ for the entire maize genome (LANDER and BOTSTEIN 1989). In this work, we used a more stringent threshold of 3.0 for declaring the existence of a QTL, and we considered LOD scores between 2.0 and 3.0 as "suggestive." For each LOD peak, we determined 1.0 LOD and 2.0 LOD support intervals (that is, the region in which the LOD score remains within 1.0 or 2.0 units of the peak).

For any point in the genome, we calculated the quantity:

$$ {}_iR^2 = 1 - \sigma^2/\sigma_{tot}{}^2. $$

where σ_{tot} is the total phenotypic variance and σ^2 is the unexplained variance for the model in which the phenotype is explained by a single QTL at the point having the maximum likelihood effect. The quantity ${}_iR^2$ is analogous to ${}_sR^2$ defined for the single-marker analysis.

In contrast to the traditional single-marker analysis (see above), the maximum likelihood approach does not omit F_3 individuals with heterozygous genotypes; this makes it possible to directly fit simultaneous multiple QTL models without omitting large fractions of the data. Accordingly multiple-QTL models were fit to the data to search for QTLs with effects that might be detected only after controlling for larger QTLs. In addition, multiple-QTL models were used to discern whether chromosomes with multiple LOD peaks contained single- or multiple-segregating QTLs (LANDER and BOTSTEIN 1989; LINCOLN and LANDER 1989; PATERSON *et al.* 1990). When adding QTLs to a model, LOD score increases of 1.0 were considered suggestive of an additional QTL and 2.0 were considered indicative.

Finally, in order to evaluate the effects of environments, we repeated the complete analyses separately for each environment. Effects of sets were again ignored in these analyses.

Analyses of genotype by environmental interaction: Two different procedures were used to assess G × E interaction. The first was based on the single-marker analysis; to study overall G × E interaction, the significance of the location by marker interaction variance was examined using an F-test (see Table 2a). The second was based on the LOD score analysis; to test whether the apparent position of a QTL differed significantly between two environments, we compared the LOD score obtained under the hypothesis of two distinct QTLs in the two environments (each located at its maximum likelihood position) to the LOD score obtained under the hypothesis of a single QTL in both environments (located at the maximum likelihood position). The test has one degree of freedom.

Relationship of heterozygosity with trait expression: The relationships of heterozygosity with trait expression were evaluated by regressing mean trait values on the percent heterozygous marker loci in the 264 BC_1 families for the two backcross populations.

Analysis of epistasis: After apparent QTLs were located, we tested each pair for possible epistasis. For example, let m_1, m_2, m_3 and m_4 denote the expected phenotypic effect of individuals in the backcross to B73 having, respectively genotypes *BB;BB, BB;BM, BM;BB* and *BM;BM*. We compared the LOD score for the maximum likelihood model allowing for epistasis (*i.e.*, allowing m_1, m_2, m_3 and m_4 to have their maximum likelihood values) to the LOD score for the

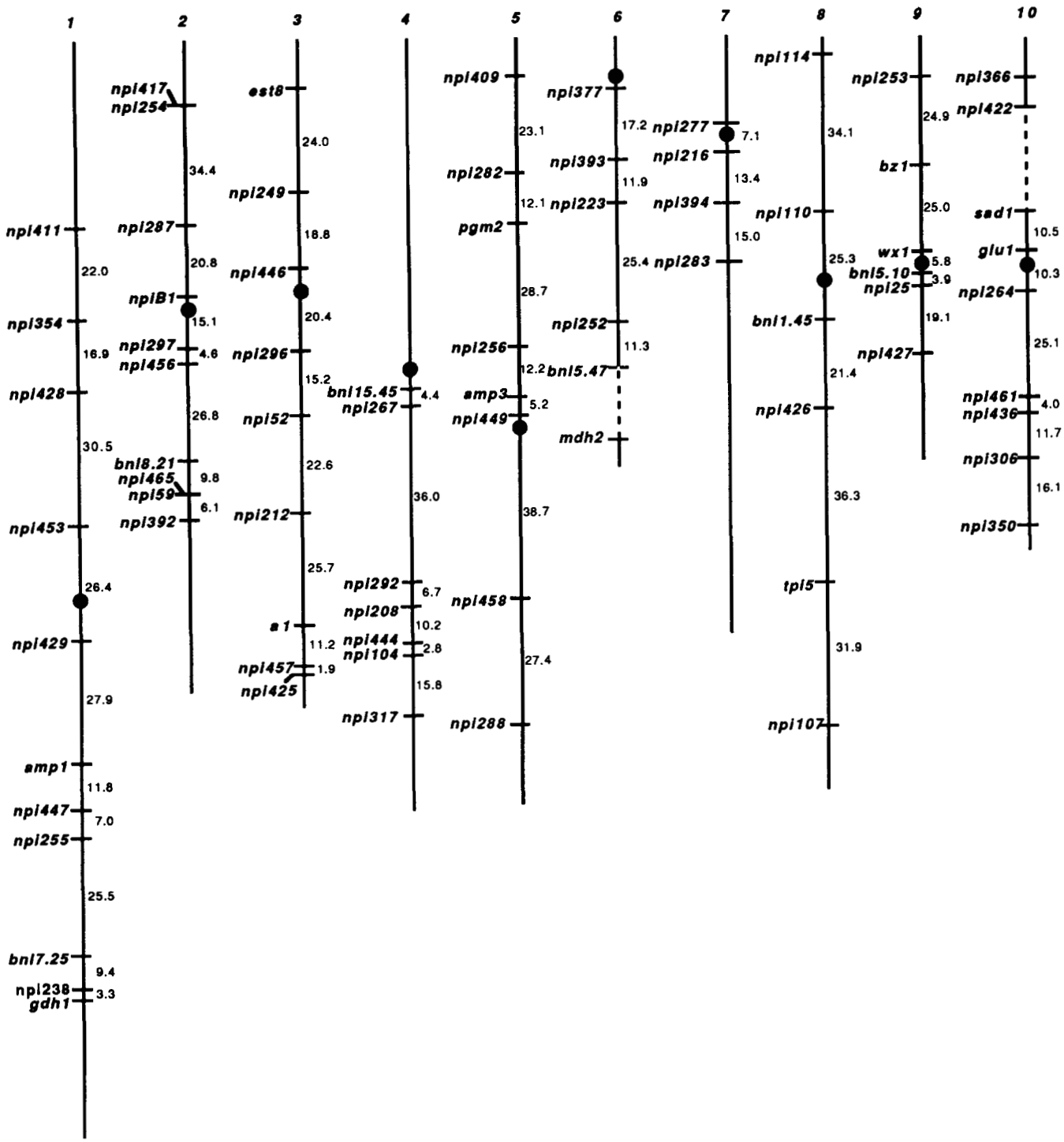

FIGURE 2.—Maize chromosome map showing locations of the 76 isozyme and RFLP markers as calculated from genetic data in this study using MAPMAKER (LANDER *et al.* 1987) as modified for F_3 family data. Isozyme loci are: *amp1*, *gdh1*, *est8*, *pgm2*, *amp3*, *mdh2*, *tpi5*, *sad1* and *glu1* The other markers are RFLPs. All markers are linked to the map with LOD scores exceeding 3.0, except where indicated by dashed lines. Centromere positions are approximate. Distances between markers are given in Kosambi centiMorgans.

maximum likelihood model subject to the constraint of no epistasis (*i.e.*, $m_1 + m_4 = m_2 + m_3$). This test has 1 d.f.

RESULTS

Genetic map: The genetic map is shown in Figure 2, with distances indicated in centiMorgans. All 76 markers were linked to the map with LOD scores exceeding 3.0. We estimate that the markers are detectably linked with 90–95% of the maize genome. Map order agreed with previously published orders [isozymes: STUBER *et al.* (1988); BNL RFLP probes: BURR *et al.* (1988); NPI RFLP probes: WEBER and HELENTJARIS (1989)] with the exceptions: (1) the two

linked probes *NPI104* and *NPI317* on the long arm of chromosome *4* were reversed in order on our map, (2) the isozyme marker *Mdh2* on the long arm of chromosome *6* and the two linked probes *NPI366* and *NPI422* on the short arm of chromosome *10* could not be assigned to any of the 10 linkage groups in our mapping analyses, and (3) the three linked probes–*BNL8.21*, *NPI59* and *NPI392*–which unequivocally mapped to chromosome *2* on our map had previously been assigned to chromosome *7* (B. BURR, personal communication). It is known that there is extensive duplication between chromosomes *2* and *7* (HELENTJARIS, WEBER and WRIGHT 1988), with the result that many probes detect fragments on both chromosomes. We presume that the probes revealed polymorphic fragments on chromosome *7* in the crosses used in the previous studies, while they revealed polymorphic fragments on chromosome *2* (and monomorphic, and thus unmappable, fragments on chromosome *7*) in the population used in our study.

QTLs for grain yield: Because most metabolic processes in the maize plant ultimately affect reproduction, it seems very likely that the inheritance of grain yield must involve multiple genetic factors. Consequently, this trait probably was the most complex of the traits evaluated in this study. It also is the most important trait for plant breeders. Consequently, analyses of grain yield will be discussed in more detail than the other traits evaluated.

The results for this trait, averaged over the six environments are shown in Figure 3. Three types of analyses were performed for mapping and ascertaining the significance of QTL effects: single-marker one-factor analysis of variance, interval mapping using LOD scores, and single-marker three-factor analysis of variance. The first two methods are directly comparable, with each using phenotypic trait means averaged over the six environments, thus ignoring set and location effects. These two methods gave broadly similar results with the following differences: (1) interval mapping tended to show higher overall significance levels; (2) interval mapping detected a significant effect for one marker lying at some distance from a QTL (*BNL8.21* on chromosome *2* in the backcross to B73) which the single-marker one-factor analysis of variance did not (but was detected by the three-factor analysis); and (3) single-marker one-factor analysis of variance detected one barely significant result (*NPI306* on chromosome *10* in the backcross to Mo17) not detected by interval mapping but this could be interpreted as a false positive. The third method (single-marker three-factor) of analysis accounted for set and location effects, thereby eliminating variance due to these causes. This approach gave results similar to the first two methods, with the exception that it was able to detect small effects on chromosome *4* in the backcross to B73 and on chromosome *6* in the backcross to Mo17 (see Figure 3).

Table 3 shows the effects of allelic substitution at the marker locus nearest the apparent QTL, computed both by the single-marker three-factor analysis of variance and by the interval mapping (see columns titled "Phenotypic effect"). For example, at the *Amp3* marker in the backcross to B73, the effect of substituting the allele from Mo17 is about 11 bushels per acre (0.69 Mg ha^{-1}). This is more than 12% of the 88.5 bushels per acre (5.55 Mg ha^{-1}) mean grain yield over the six environments.

Because the three methods of analyses provided similar results, we will focus on the LOD score analysis because it provides a simple picture of the likely location of the QTLs. Markers showing significant association with yield in at least one backcross were found on all 10 chromosomes. In the B73 backcross population, the long arm of chromosome *1* and the centromeric regions of chromosomes *2* and *5* showed highly significant effects with LOD scores greater than 6.0, the centromeric regions of chromosomes *7* and *9* showed LOD scores greater that 4.0, and the centromeric region of chromosome *10* showed a LOD score greater than 3.0. In the Mo17 backcross, the long arms of chromosomes *3* and *4* and the centromeric region of chromosome *5* showed highly significant effects with LOD scores greater than 6.0, the proximal region of the long arm of chromosome *1* showed a LOD score greater than 4.0, and the centromeric regions of chromosomes *7*, *8*, *9* and *10* showed LOD scores exceeding 2.77. Thus, the backcross to B73 showed at least six QTLs and the backcross to Mo17 showed at least eight QTLs for grain yield.

Separate analyses for each of the six individual environments based on interval mapping using LOD scores are shown in Figure 4. In spite of the large variation in mean grain yields among environments (Table 1), results for each environment are remarkably consistent, particularly in those regions for which the LOD scores (for means over environments) were greater than 3.0. (Deviations from this consistency in the backcross to B73 were found on chromosome *1* in the region between markers *NPI453* and *Amp1* and on chromosome *10* between markers *Glu1* and *NPI461*. Similar exceptions occurred in the backcross to Mo17 on chromosome *2* in the region between markers *NPIB1* and *BNL8.21*, on chromosome *4* between markers *NPI267* and *NPI292*, and on chromosome *10* between *Sad1* and *NPI264*.) Statistical tests based both on traditional analyses of variance and LOD-score analyses confirmed the general lack of evidence for significant QTL-by-environment interaction.

Finally, no convincing evidence for epistasis was found: about 1% of the pairwise tests were significant

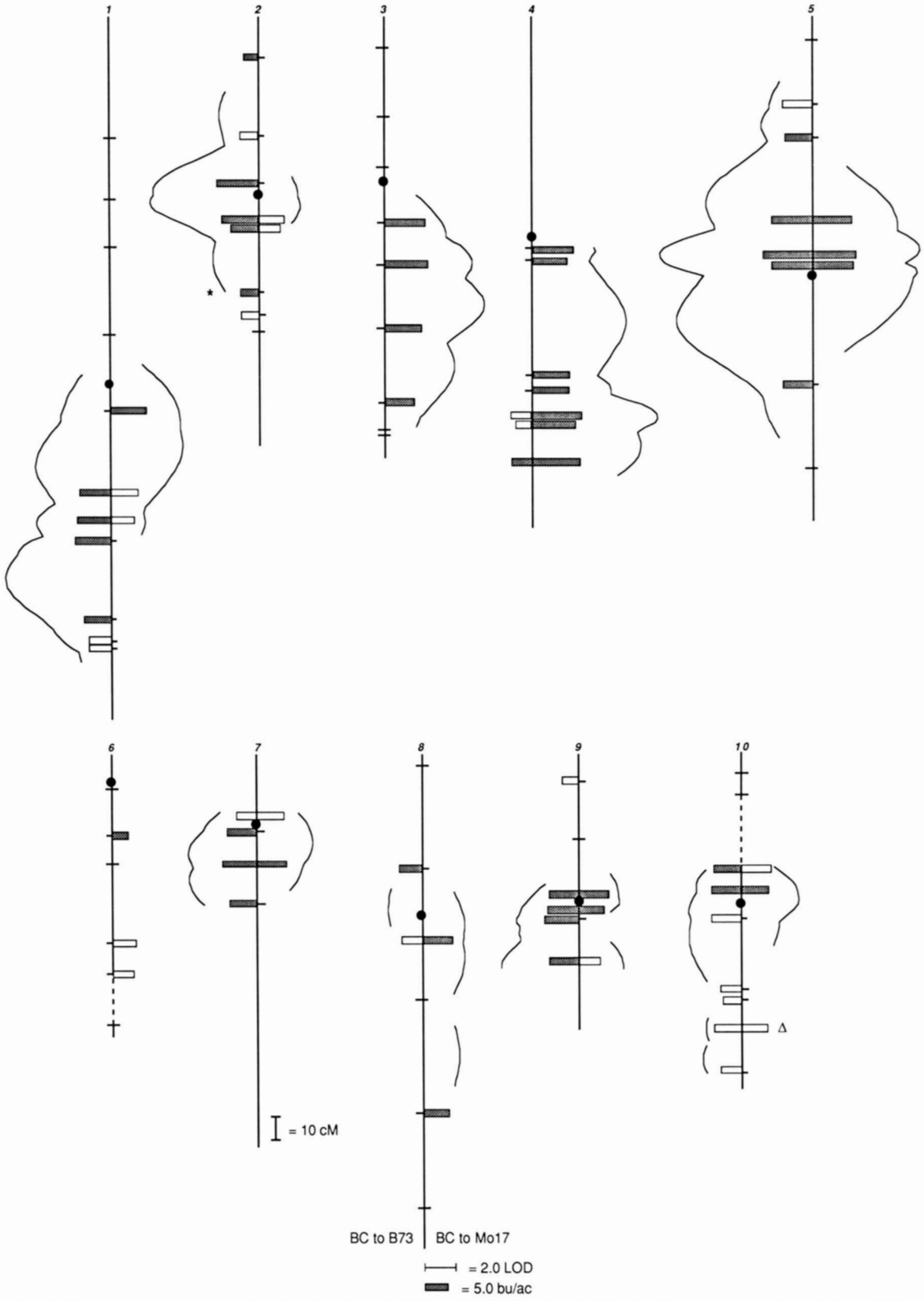

FIGURE 3.—Maize chromosome map showing location of QTLs affecting grain yield in the backcrosses to inbred lines B73 and Mo17 evaluated over six environments. The map summarizes the results of three analyses: interval mapping using LOD scores, single-marker one-factor analysis of variance, and single-marker three-factor analysis of variance [accounting for set and location (environment) effects]. Interval mapping is represented by QTL likelihood plots showing LOD score curves exceeding the threshold of 2.0. Single-marker one-factor analysis is not explicitly represented because it agreed extremely well with the interval mapping analysis; the few discrepancies are indicated by an asterisk at the one locus at which the LOD score was significant but the one-factor analysis of variance was not, and by a triangle at the one locus at which the opposite was found. Single-marker three-factor analysis is shown by bars protruding from the chromosome, whose length indicates the estimated phenotypic effect of substituting an allele at the QTL in the vicinity of the marker. Bars are shaded light and dark gray to indicate significant association exceeding the 0.01 and 0.001 levels, respectively. For all analyses, results from backcrosses to B73 are shown on the left and results from backcrosses to Mo17 are shown on the right of the vertical lines representing each chromosome.

TABLE 3

Percentage of variance (R^2) and phenotypic effect attributable to individual QTLs affecting grain yield in backcrosses to B73 and Mo17, for single-marker and interval mapping analyses

	Single-marker analyses			Interval-mapping analyses			
				Single-QTL			*N*-QTLs
Chr. marker	${}_iR^2$ (%)	${}_sR^{*2}$ (%)	Phenotypic effect (bu./acre)	LOD	${}_iR^2$ (%)	Phenotypic effect (bu./acre)	Phenotypic effect (bu./acre)
Backcross to B73:							
1 NPI255	12.8	24.0	8.07	6.91	15.1	10.40	8.72
2 NPIB1	15.1	20.0	8.80	6.63	13.3	9.72	9.76
5 Amp3	21.3	34.9	10.85	9.73	18.0	11.30	10.40
7 NPI216	8.4	15.8	6.60	4.44	8.8	7.98	6.74
9 NPI427	10.7	23.0	7.65	4.80	10.3	8.70	7.56
10 NPI264	8.1	17.8	6.65	3.16	6.2	6.52	3.54
All 6 QTLs: ${}_iR^2 = 60.9\%$	Total effects = 46.72 bu./acre						
Backcross to Mo17:							
1 NPI429	9.7	16.8	8.41	4.78	9.5	9.50	7.71
3 NPI212	9.3	19.2	8.01	6.53	14.4	12.38	11.57
4 NPI444	15.4	26.3	10.86	8.01	13.9	11.34	10.55
5 Amp3	13.0	29.5	9.71	6.86	12.9	13.72	8.42
7 NPI216	8.7	25.1	7.86	3.31	6.4	8.02	5.38
8 BNL1.45	5.9	15.7	6.33	2.73	5.8	7.68	6.70
9 NPI427	7.6	15.0	7.23	2.97	5.6	7.52	7.60
10 Glu1	7.7	21.0	7.60	3.56	6.5	7.06	4.68
All 8 QTLs: ${}_iR^2 = 59.1\%$	Total effects = 62.61 bu./acre						

Descriptions of R^2 values are given in MATERIALS AND METHODS. Under interval mapping, *N*-QTLs column refers to a multiple QTL model in which phenotypic effects were estimated simultaneously for all QTLs.

at approximately the 0.01 significance level.

QTLs for other traits: The results of LOD score analyses for each chromosome are shown for plant height, ear leaf area, days to tassel, grain moisture, and ears per plant in Figure 5. These were computed on the mean trait values over all environments (see Table 1) in which the trait was evaluated.

A minimum of three QTLs, on chromosomes *1*, *9* and *10*, were detected for plant height in the backcross to B73. For this trait, at least five QTLs were found on chromosomes *2*, *3*, *4*, *7* and *8* in the backcross to Mo17. For ear leaf area, QTLs were detected only on chromosomes *1*, *2* and *9* in the backcross to B73. However, at least six QTLs were detected on chromosomes *2*, *3*, *4*, *5*, *8* and *10* in the backcross to Mo17.

A minimum of four QTLs associated with days to tassel were noted in the B73 backcross on chromosomes *1*, *3*, *6* and *8*; in the Mo17 backcross at least three QTLs were detected on chromosomes *1*, *8* and *9*. Grain moisture showed associations with a minimum of four QTLs on chromosomes *1*, *2*, *8* and *10* in the backcross to B73; also at least four QTLs were noted for this trait in the backcross to Mo17 on chromosomes *1*, *2*, *5* and *8*. Only two QTLs were detected for ears per plant in each of the backcrosses: on chromosomes *3* and *6* in B73, on *7* and *8* in Mo17.

Proportions (R^2) of variances and phenotypic effects attributed to grain yield QTLs: For the single-marker three-factor analyses, ${}_sR^2$ and ${}_sR^{*2}$ were computed only for individual markers nearest the QTL identified using LOD scores. However, for the interval mapping analyses, ${}_iR^2$ values were computed for the presumed location of each QTL as determined by the peak LOD score. All R^2 values were converted to percent (by multiplying by 100) for discussion purposes (Table 3). The ${}_sR^2$ values represent the proportions of the phenotypic variance among backcross families accounted for by the respective QTLs and ranged from 5.9 for marker *BNL1.45* in the Mo17 backcross to 21.3 for *Amp3* in the B73 backcross. The ${}_iR^2$ values were 5.8 and 18.0 for those two markers, respectively. Table 3 shows that the magnitudes of the ${}_sR^2$ and the ${}_iR^2$ values were quite similar for all of the markers. Multiple QTL analyses could be done only using interval mapping and these results showed that the six QTLs (associated with grain yield) accounted for about 61% of the phenotypic variance in the B73 backcrosses and the eight QTLs (associated with grain yield) accounted for 59% of the phenotypic variance in the Mo17 backcrosses.

The ${}_sR^{*2}$ values, which presumably reflect the proportion of genetic variance accounted for by the QTLs in the vicinity of the markers ranged from 15% to nearly 35%. Although each marker listed is on a different chromosome and would segregate independently, obviously the ${}_sR^{*2}$ values are not independent because their sums were greater than 100% in each backcross. However, when the relative contributions

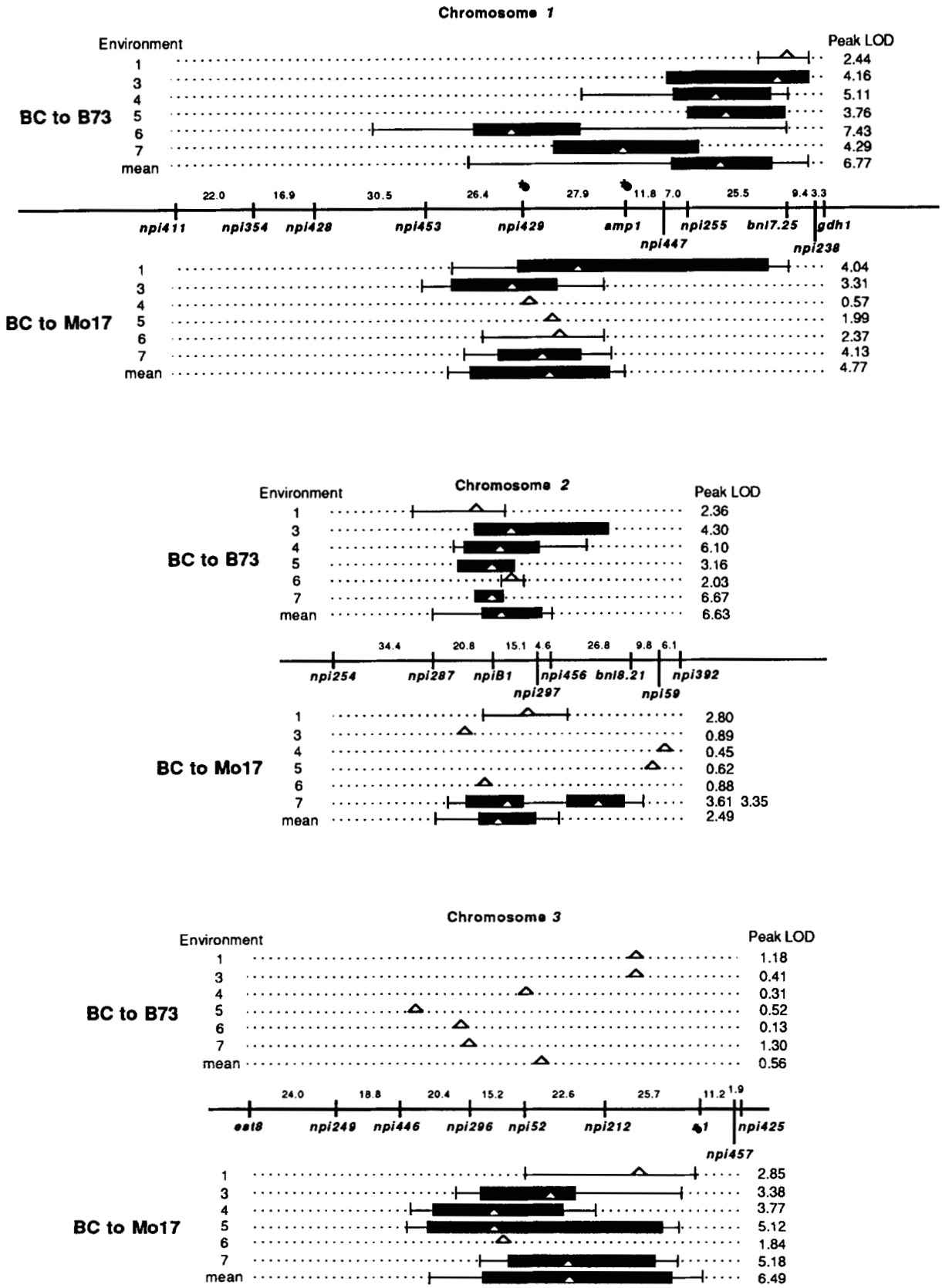

FIGURE 4.—Likelihood plots of LOD scores for grain yield in the backcrosses of F_3 lines to B73 and Mo17. Results depicted are from evaluations in each of the six environments (environments 1, 3, 4, 5, in North Carolina; 6 in Illinois, and 7 in Iowa) and are shown for each chromosome. The horizontal line in the center of each plot shows the markers and map distances for that chromosome. Dark shaded bars represent LOD scores greater than 3.0 with the extensions representing LOD scores >2.0 and <3.0 The values on the right of each plot are the maximum LOD scores and the Δ's designate the map locations of the maximum scores which are the most likely locations for the putative QTLs. Asterisks designate markers which showed significant marker (QTL) by interaction effects from analyses of variance.

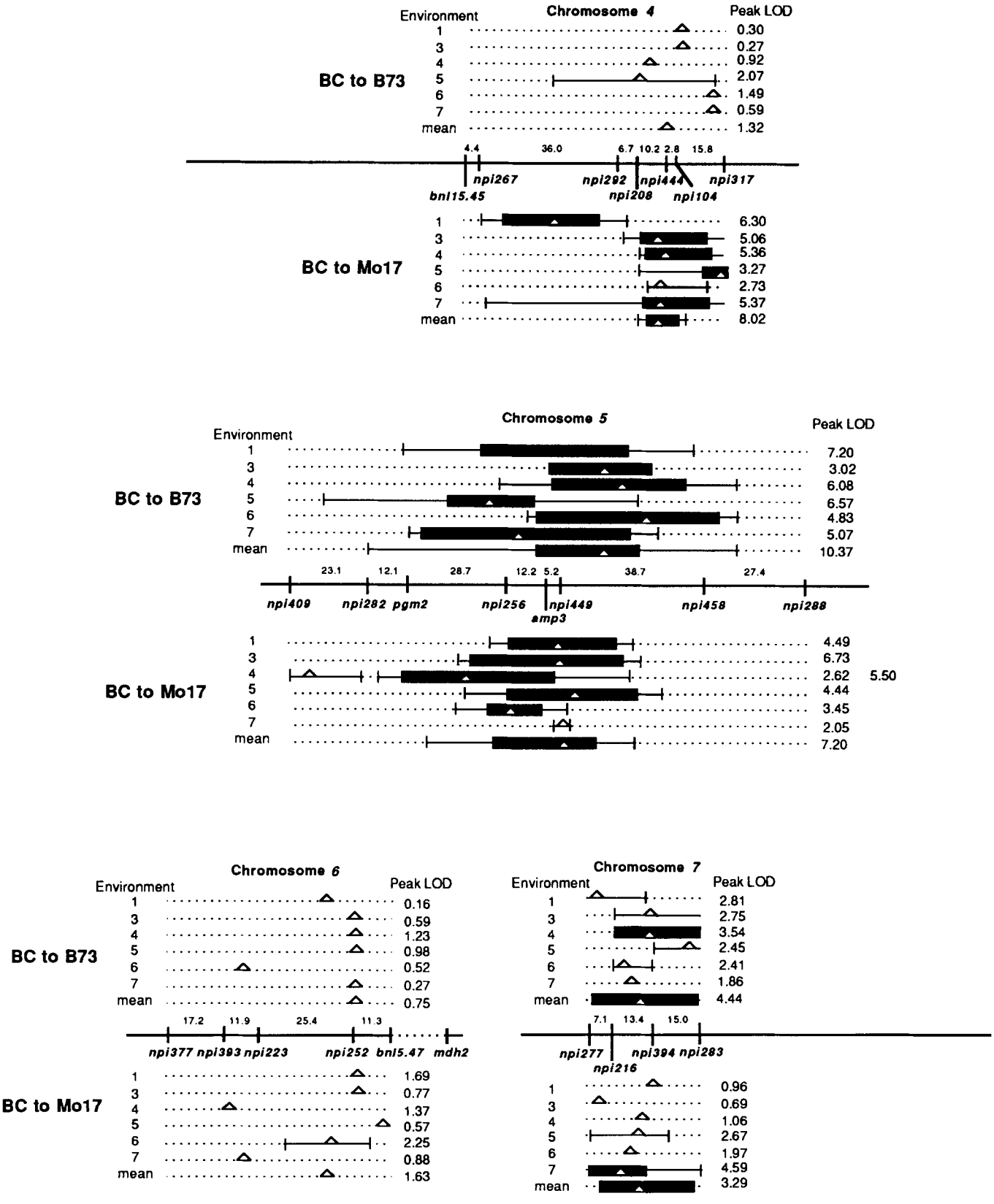

FIGURE 4.

of the QTLs were considered, they were similar for ${}_sR^2$ and ${}_sR^{*2}$.

The phenotypic effects attributed to the QTLs in the vicinities of the markers were very similar for both the single-marker and the interval-mapping analyses. The range for the interval-mapping analyses was from

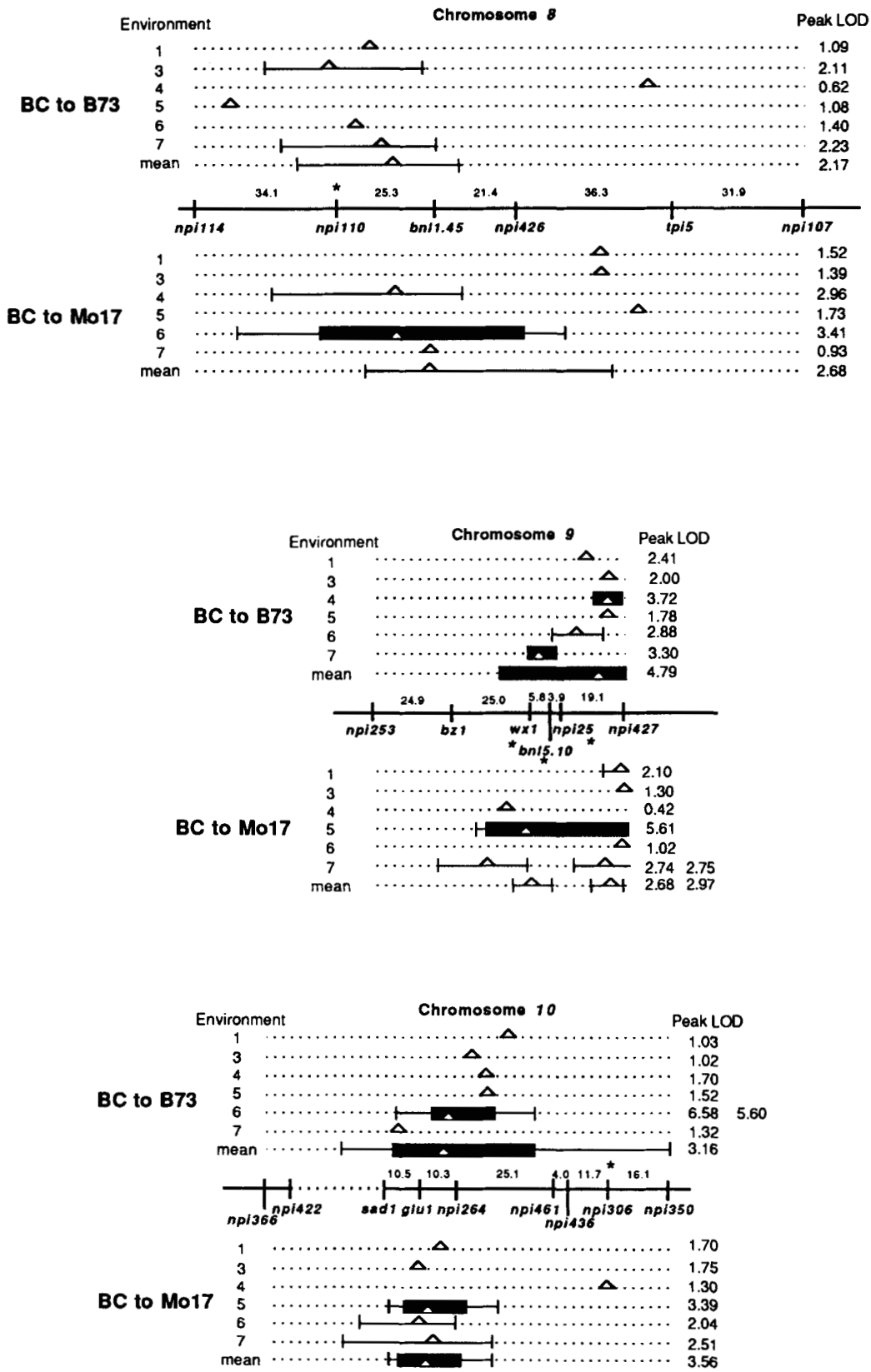

FIGURE 4.

6.5 bu./acre at the *NPI264* marker on chromosome *10* in the backcross to B73 to 13.7 bu./acre at the *Amp3* marker on chromosome *5* in the backcross to Mo17. The total phenotypic effects from the multiple QTL analyses were 46.7 bu./acre for the six QTLs in the backcross to B73 and 62.6 bu./acre for the eight QTLs in the backcross to Mo17.

Relationship of heterozygosity to trait expression:

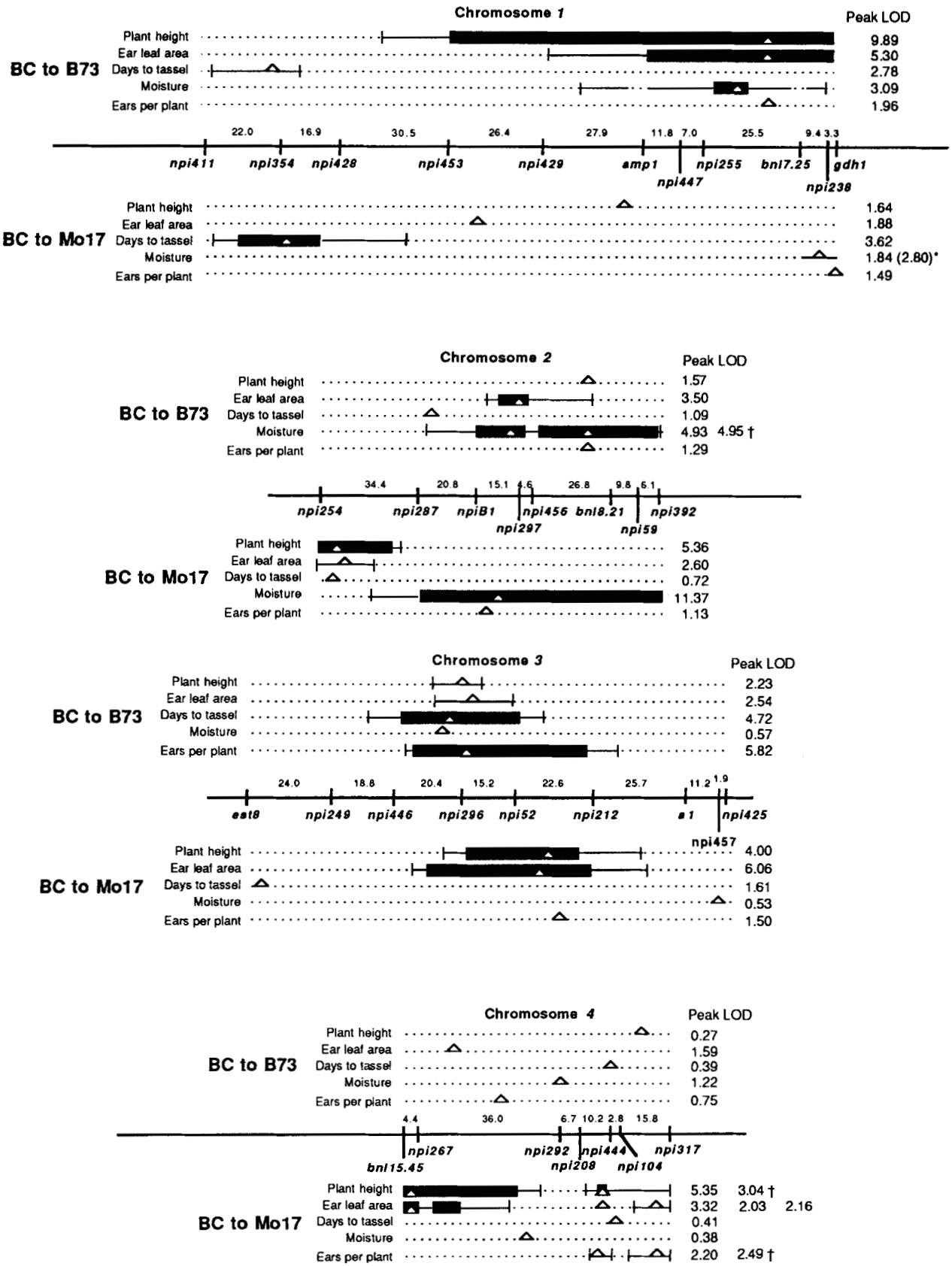

FIGURE 5.—Likelihood plots of LOD scores for plant height, ear leaf area, days to tassel, grain moisture, and ears per plant in the backcrosses of F_3 lines to B73 and Mo17. Results depicted are from evaluations over environments (see Table 1 for number of environments for each trait). Presentation of results is in the same format as for Figure 4. Asterisk indicates LOD score after substracting variation caused by one or more major QTLs elsewhere in the genome. † indicates cases where multiple-QTL models did not rule out the possible existence of multiple-linked QTLs.

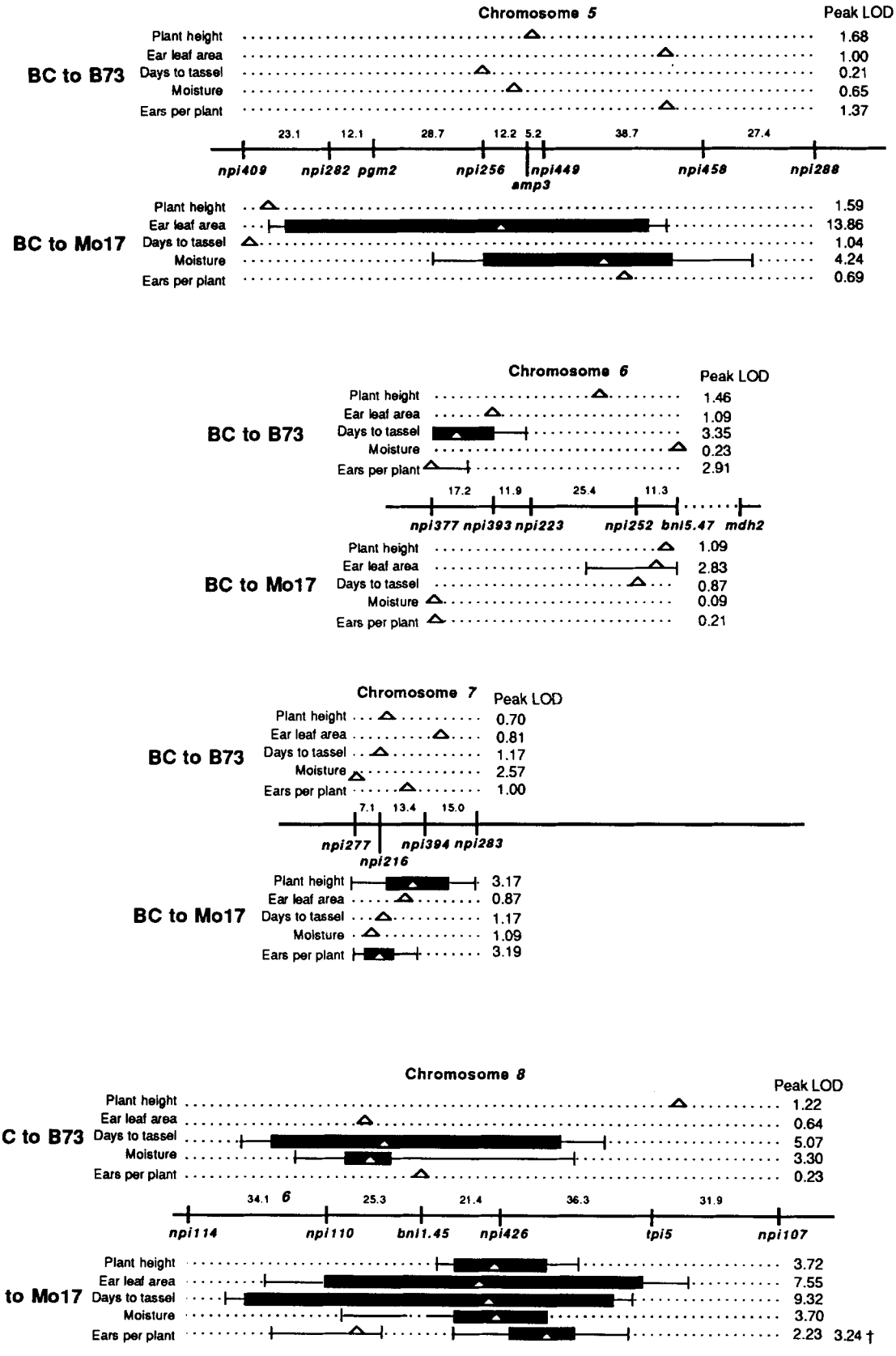

FIGURE 5.

The importance of heterozygosity to trait expression was obtained by examining the mean level of trait performance observed for a given percent of heterozygous loci. For grain yield, there was a highly significant relationship with heterozygosity as evidenced by the r value of 0.68 in the backcrosses to both B73 and

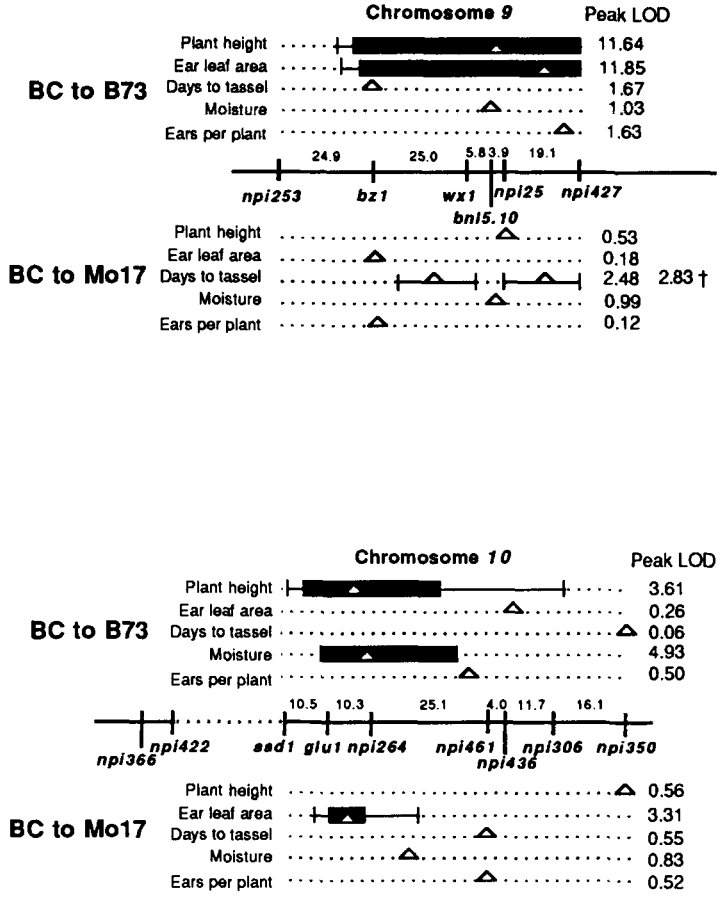

FIGURE 5.

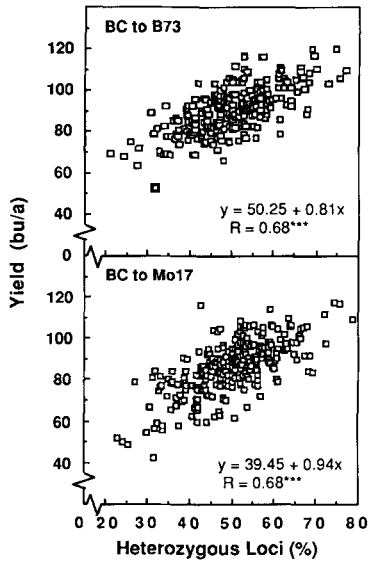

FIGURE 6.—Plots showing associations of grain yield with percent of heterozygous loci for the 264 backcrosses to B73 and 264 backcrosses to Mo17. Regression and correlation statistics are shown also.

Mo17 (see Figure 6). Other traits also showed a significant relationship between mean trait performance and number of heterozygous loci, but the r values were generally lower, ranging from 0.06 for ears per plant to 0.50 for plant height in the backcross to B73 (Table 4).

TABLE 4

Correlation coefficients (r) between several quantitative traits and the percent heterozygous marker loci in 264 BC_1 families for the two backcross populations

Trait	Backcross to B73	Backcross to Mo17
Grain yield	0.68***	0.68***
Ears per plant	0.06	0.21***
Ear height	0.26***	0.22***
Plant height	0.50***	0.31***
Leaf area	0.37***	0.47***
Days to tassel	−0.14*	−0.11
Grain moisture	0.19**	−0.08

*, ** and *** indicate a significant correlation at the 0.05, 0.01 and 0.001 levels, respectively.

DISCUSSION

Comparison of methodologies: The present study provided an opportunity to compare different methodologies for QTL mapping. Traditional single-marker one-factor analysis of variance and the more recently described interval-mapping approach (LANDER and BOTSTEIN 1989) are directly comparable, inasmuch as both involved no correction for set and

location effects. Although the two methods yielded virtually identical results in terms of QTLs identified, the interval mapping approach appeared to offer some advantages, particularly for the type of experimental materials evaluated in this study. For example, interval mapping uses information on flanking markers of putative QTLs, which may provide more power than the single-marker method for detecting QTLs. In addition, interval mapping provides information about the likely location of the QTL, even estimating the maximum likelihood position under the assumption that there is a single QTL in the region (which assumption, of course, may or may not be true for very complex traits such as yield). Interval mapping also allows ambiguous or missing data, whereas (for the experimental design used in this study) the single-marker analysis excluded F_3 individuals that were heterozygous at a locus because their progenies were mixtures of two types (see MATERIALS AND METHODS); this made it possible in this study to use interval mapping to construct multilocus models without cumulative loss of individuals from the dataset. Because interval mapping represents a generalization of the traditional single-marker one-factor analysis, it is not surprising that it offered some advantages without significant losses.

The single-marker three-factor analysis differed by allowing for set and location effects. Although the results were broadly similar to those above, the method provided increased power and allowed detection of QTLs in two additional regions. This third method found weak but significant evidence for QTLs for yield on chromosome *4* in the B73 backcross and on chromosome *6* in the Mo17 backcross (Figure 3), whereas the interval mapping approach showed LODs (1.32 and 0.75, respectively) which were below threshold levels and were only suggestive of QTLs on these chromosomes. All major QTLs with LODs of 3.0 or greater were detected by all analytical methods, however.

Analogous interval mapping analysis allowing for set and location effects was not conducted, due to the limitations in the current MAPMAKER-QTL software package. However, there is no fundamental limitation to including these effects in the likelihood model–which should combine the advantages of the single-factor method with the interval mapping method. (E. S. LANDER hopes to undertake these modifications in the near future.)

QTLs for yield: QTLs affecting grain yield were detected in at least one of the two backcrosses on all 10 maize chromosomes. The finding of a large number of QTLs is not surprising in view of the complex nature of the phenotype. The effects associated with a single QTL ranged as high as 13.7 bu./acre for the QTL in the vicinity of the *Amp3* marker on chromosome *5* in the backcross to Mo17. It should be noted that in the backcross to B73, the six QTLs associated with grain yield showed an overall phenotypic effect of 46.7 bu./acre, which is more than 50% of the mean for that backcross population. These six QTLs also accounted for more than 60% of the total phenotypic variation. The backcross to Mo17 showed a total phenotypic effect of 62.6 bu./acre for eight QTLs which also accounted for nearly 60% of the phenotypic variation.

It is intriguing to compare the present findings with our results from 20 other crosses (15 involving elite inbred lines, five involving elite lines with some introgression of exotic material from Latin American maize germplasm) that have been studied in our research program in Raleigh, North Carolina (EDWARDS, STUBER and WENDEL 1987; STUBER, EDWARDS and WENDEL 1987; ABLER, EDWARDS and STUBER 1991; EDWARDS *et al.* 1992; STUBER 1992; our unpublished data). Although some of these crosses have been studied with only a limited number of genetic markers, some consistent patterns are beginning to emerge. For example, 16 of the 20 populations studied have shown genetic factors significantly associated with yield in the interval flanked by markers *Pgm2* and *Amp3* on chromosome *5*. Similarly, QTLs for grain yield were found on the long arm of chromosome *1* in 13 of the 18 crosses in which linked markers were scored, and on chromosome *9* near *Acp1* in 13 of 17 crosses in which nearby markers were scored. Of course, a given quantitative trait locus–no matter how important its effect on grain yield–can be identified only in crosses in which the parents have different alleles. We will discuss the comparison of these crosses more fully elsewhere.

QTLs for other traits: For the other traits studied, fewer QTLs were identified than for grain yield. However, for traits that are associated with overall plant vigor, such as ear leaf area, plant height and ear height, the QTLs showed many similarities with those for grain yield. With very few exceptions, QTLs for plant height and ear leaf area were found in the vicinity of those associated with grain yield (see Figure 5). The correlation between QTL positions for these traits may result either from pleiotropic effects of single QTLs or to linked QTLs. We tend to favor the former notion, but recognize that the alternatives cannot be distinguished without finer mapping of the QTLs.

Although ears per plant is a component of grain yield, there were few similarities in the location of the QTLs for the two traits. This is not surprising: both of the parental lines are primarily single-eared, there was little variability for number of ears in the progenies, and this limited variation was not associated with the variation in grain yield (data not shown).

Heterosis: Our crosses were designed to maximize the ability to detect QTLs contributing to heterosis: we employed F_3 individuals so as to increase recombinational segregation of linked QTLs relative to using F_2 individuals; and we performed separate backcrosses to each of the parental strains, so as to more accurately resolve the additive and dominance effects of QTLs relative to the situation of simply selfing the individuals. The results showed a striking pattern: (1) whenever a QTL for yield was detected, the heterozygote had the higher phenotype (with the sole exception of *NPI253* on chromosome *9* in the back cross to B73). (2) QTLs for yield tended to occur in the same locations in both backcrosses. Significant QTLs were found on chromosomes *1*, *5*, *7*, *8*, *9* and *10* in both backcrosses, while chromosomes *2*, *3* and *4* showed significant effects only in one backcross (although the QTL on chromosome *2* in the backcross to B73 was associated with a suggestive, subthreshold effect in the other backcross). Thus, the majority of QTLs were associated with overdominance (*i.e.*, a higher yield in the heterozygote than in either homozygote), suggesting that these regions may play especially important roles in the phenomenon of heterosis.

As CROW (1952) has pointed out, heterosis may result from either true overdominance (single loci at which the heterozygous phenotype exceeds that of either homozygote) or from pseudo-overdominance (linked loci with advantageous alleles in repulsion phase). Our results cannot distinguish these possibilities. Indeed, they will be difficult to distinguish without extensive recombinational separation of linked loci, and perhaps impossible without cloning of the QTLs so as to identify their effects directly.

The overall effect of heterosis also can be seen by examining the correlation between a phenotypic trait and the proportion of heterozygous markers. This correlation is very high (about 0.68 in each backcross) for grain yield, while it is considerably lower for most of the other traits (Figure 6 and Table 4). This observation is consistent with our results (and our prior expectations) that grain yield is affected by more QTLs than the other traits. A trait controlled by a single locus should show little correlation between phenotype and overall heterozygosity across the genome, a trait controlled by two loci should show somewhat higher correlation, and a trait controlled by many loci across the genome should show the highest correlation. (If we assume that a trait is controlled by k loci having equal and purely heterotic effects and having heritability h, it can be shown that the correlation of phenotype with overall proportion of heterozygous genetic markers is proportional to the square root of hk.)

G × E interaction: The limited evidence for interaction of environments with QTLs (see Figure 4) is surprising for several reasons: (1) when maize traits (such as grain yield) have been evaluated *per se* in several diverse environments, genotype by environment interaction usually has been found to be significant (MOLL *et al.* 1978), (2) because this study used six environments in three states (four in North Carolina, one in Illinois, and one in Iowa), the diversity among environments was expected to be greater than found in most documented studies of maize, and (3) in the analyses of variance (see Table 2) for this study, the location by set ($L \times S$) component of variance was usually significant, particularly for grain yield (data not shown). The $L \times S$ component of variance should be analogous to the traditional genotype-by-environment interaction variance reported in many maize studies. Because of the similarity of LOD scores across environments for traits such as grain yield (see Figure 4), particularly when the scores are greater than 4.0, we believe that it may be possible to reliably detect major QTLs in relatively few environments, possibly no more than two or three.

CONCLUSIONS

Identification of QTLs affecting agronomically important traits in maize is a key step in using molecular genetics for plant improvement and in understanding genetic phenomena in plants (such as heterosis and G × E interaction). Here, we have mapped the positions of QTLs, and evaluated the phenotypic effects associated with these QTLs, for several quantitative traits in a large study designed to shed light on genetic mode of action. From the standpoint of detecting QTLs, we found that two analytical methods, single-marker and interval mapping, provided virtually identical results in backcross populations developed from the cross between inbred lines B73 and Mo17. We also found that QTL alleles causing high grain yield show a strong tendency toward dominance and usually overdominance.

QTLs identified in this cross between B73 and Mo17 may depend on the particular design of the cross. In our study, QTL effects were measured in the genetic background of backcrosses (*i.e.*, 75% recurrent parent). Results for other types of experimental materials (such as F_2 plants, F_3 families, or testcrosses) may conceivably be quite different. Nonetheless, we find intriguing the high correlations between the regions identified in this study and regions identified in other studies in our research program. Also, the relatively minor evidence in this study for marker (or QTL) by environment interaction was somewhat surprising and may differ from results in other studies and for other traits (PATERSON *et al.* 1991; BUBECK *et al.* 1992).

Detailed understanding of QTL effects will now require fine-mapping studies such as described by

PATERSON *et al.* (1990). Several regions have been identified for such analyses, such as the region in the vicinity of the centromere on chromosome *5* and the long arm of chromosome *4*.

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